A PRELIMINARY REPORT ON THE ACCUMULATION OF DDT IN FISH FROM THE MUSKOKA LAKES SYSTEM

ONTARIO WATER RESOURCES COMMISSION
December, 1966

A PRELIMINARY REPORT ON THE ACCUMULATION OF DDT IN FISH FROM THE MUSKOKA LAKES SYSTEM

Biology Branch
Chemistry Branch II
DIVISION OF LABORATORIES

December, 1966

[illegible]
[illegible]
[illegible]

[illegible]
[illegible]
[illegible]

[illegible]
[illegible]
[illegible]
[illegible]

[illegible]

TABLE OF CONTENTS

SUMMARY AND RECOMMENDATIONS

The DDE concentrations in the fish considered in this report were generally of an extremely high level Furthermore, the projected concentrations of DDT in the gonads of five female lake trout included in the study were such that total mortality of fry hatching from these eggs would have been likely. Levels of DDE in the flesh (muscle) of the lake trout, ranging between 4.3 ppm and 110 ppm, raises the question of the suitability of these fish for human consumption.

The following recommendations are made:

(1) In view of the serious consequences which may be related to the established accumulation of DDT and its metabolites in fish, the use of this insecticide should be prohibited before the 1968 spraying season

(2) An expanded sampling programme should be established to ascertain the DDT and DDE contents in populations representing various trophic levels in the Muskoka Lakes. To this end, various species of fish, spawning lake trout and fertilized eggs, mud and water samples were collected in November, 1967. Currently, hatchability studies are in progress to evaluate the effects of the DDT content of the fish eggs on the fecundity of the lake trout.

(3) The judgement of a competent medical authority should be obtained to determine the acceptability of lake trout, whitefish and other species from the Muskoka Lakes for human consumption

(4) The Food and Drug Directorate of the Department of National Health and Welfare should be requested to provide residue tolerances for the more commonly used or hazardous pesticides in fish

A PRELIMINARY REPORT ON THE ACCUMULATION OF DDT IN FISH FROM THE MUSKOKA LAKES SYSTEM

INTRODUCTION

Widespread concern has developed as a result of relationships which appear to exist between the use of stable insecticides for controlling insect pests and declines in fishery production owing to translocation of these compounds to the aquatic environment.

The acute toxicity of the insecticide DDT to fish and other aquatic life has been well documented by workers such as Henderson et al. (1959), Ide (1957), and Cope (1961). That DDT might be implicated in reproductive losses in fish populations was not established until Burdick et al. (1964) in New York State reported heavy mortality of lake trout fry when the ether extract of the eggs reached 2.9 ppm or above. Anderson and Everhart (1966) were led to suspect that the use of DDT for biting fly control was responsible for a decline of a landlocked salmon fishery in Sebago Lake, Cumberland County, Maine, and their work records the accumulation of DDT and its metabolite DDE in body tissue, liver, gonads and fat content in fish within different age groups.

In Ontario, substantial quantities of chlorinated hydrocarbon pesticides are used to augment agricultural production and to provide high quality foodstuffs that will gain ready consumer acceptance. Two of the most striking examples where large quantities of insecticides are used annually are the Holland Marsh area, noted for its vegetable production, and the

tobacco-growing region centered on the counties of Elgin, Oxford, Norfolk and Brant.

In addition, substantial aircraft spraying of the insecticide DDT is carried out in central and northern Ontario each year for control of adult blackflies and mosquitoes.

The information provided by Burdick and his co-workers (1964) and other studies indicating the accumulation of DDT by fish (Cope, 1961; Mack et al., 1964) led to a discontinuance of direct applications of DDT to water in Ontario, which was possible since a permit system regulating the use of aquatic control agents was already in effect. Efforts were commenced to acquire samples of fish from the aforementioned areas in order to analyze for the presence of this insecticide and a start was made on sampling fish from the lower Great Lakes system. Facilities at the OWRC laboratory were used to analyze for the presence of DDT in water, fish and aquatic invertebrate life.

Priority was given to fish collected from the Muskoka Lakes System because of the obvious decline of the lake trout in these lakes in recent years and since sport fishing is an important mainstay of the tourist economy in central and northern parts of the province. It was felt that the information derived from the Muskoka Lakes would provide a satisfactory indication of whether or not concern on a more general scale would be warranted, since the degree of development in that area from the standpoint of the tourist industry is relatively heavy.

This report provides the results of DDE analyses (a DDT metabolite) for lake trout obtained from Lake Muskoka, Lake Joseph and Lake Rosseau and considers the significance of these results in the light of knowledge which is available from other sources.

THE MUSKOKA LAKES

The Muskoka Lakes system is comprised of Lake Muskoka, Lake Joseph and Lake Rosseau, stretching north-west some 28 miles from Gravenhurst to Rosseau at the north end of Lake Rosseau, and Port Cockburn at the north end of Lake Joseph. The shoreline of these lakes is intensively developed for summer cottage use as well as commercial resort operations.

Fish species of significance to sports fishermen in the Muskoka lakes are lake trout, Salvelinus namaycush; smallmouth bass, Micropterus dolomieui; speckled trout, Salvelinus fontinalis; rainbow trout, Salmo gairdneri; yellow walleye, Stizostedion vitreum; smelt, Osmerus mordax; sunfish, Lepomis gibbosus; yellow perch, Perca flavescens; and whitefish, Coregonus clupeaformis.

In recent years, there has been an obvious decline in the lake trout fishing in Lake Muskoka and Lake Rosseau. Surveys conducted by fish and wildlife personnel of the Department of Lands and Forests in 1960 and 1961 indicated no natural reproduction of lake trout and virtually no survival of planted yearlings. A planting of 6,000 two-year old lake trout in 1965 was considered to be successful since it more than doubled the lake trout population in Lake Muskoka. Because of this success a similar planting was repeated in 1967.

Unlike Lake Rosseau and Lake Muskoka, a satisfactory native lake trout fishery has prevailed in Lake Joseph.

Considerable aerial spraying for mosquito and blackfly control has been carried out in the Muskoka area since DDT became popular for insect control after World War II. Commercial pest control operators contract their services to summer resorts, youth camps, golf clubs and cottagers in order to provide some measure of localized relief from the biting fly problem. Thus, most of the spraying has been completed in close proximity to shoreline areas.

Exposed granitic outcrops and shallow, sandy soil on top of the bedrock is characteristic of the entire Muskoka area. This condition provides a poor retention capacity for pesticides applied over the land. Under these circumstances, and considering the residual nature of DDT, contamination of the water through run-off and percolation was considered a serious possibility.

FIELD METHODS

Information was obtained from one of the most active commercial air spray companies in the Muskoka District, concerning those areas where spraying activities had been concentrated in the summer of 1966. This information was utilized to fix the location of gill nets which were set during the fall season to collect fish for DDE analyses. Personnel of the Fish and Wildlife Branch of the Department of Lands and Forests (Parry Sound District) collected lake trout and whitefish in the vicinity of Norway Point and Treasure Island on Lake Rosseau and near Bracebridge and Beaumaris Island on Lake Muskoka. These fish were frozen and transported to Toronto where they were held in cold storage until the analyses were completed in November of 1967.

LABORATORY PROCEDURES

In preparation for the analytical procedure the fish were thawed under refrigeration and selected tissues were dissected, ground and then refrozen to await further processing. The tissue sections included a skinned portion of muscle taken between the mid-dorsal and lateral lines, the liver including the gall bladder and bile, and whole gonads.

ANALYSIS OF DDT-TYPE PESTICIDES IN FISH

DDT, (I) and its derivatives, DDD (II) and DDE (III)* belong to a group known as chlorinated hydrocarbon pesticides. They are highly stable compounds and consequently, very persistent (Cope,1961; Bridges et al, 1963). Under certain conditions however, these materials can be metabolized by living organisms. For example, DDT in fish is broken down enzymatically mainly to DDE and DDD. On the other hand, micro-organisms, under anaerobic conditions, metabolize DDT and DDD (Hill and McCarty, 1957). In addition, there is evidence of other types of breakdowns, e.g. upon storage of tissue

---

(I) p,p'-DDT: 1,1,1.- trichloro-2,2-bis(p-chlorophenyl)ethane.
(II) p,p'-DDD: 1,1.-dichloro-2,2-bis(p-chlorophenyl)ethane.
(III) p,p'-DDE: 1,1.-dichloro-2,2-bis(p-chlorophenyl)ethene.

and blood specimens at subzero temperatures it is found that DDT "disappears" (Ecobichon and Saschenbrecker, 1967). In view of this it is clear that analytical specimens would contain mixtures of the various DDT metabolites. Although it is possible to analyze for all of the metabolites, it is customary to analyze for only the major components, DDT, DDE and DDD. Frequently the results are expressed in terms of p,p'-DDT', the major component in technical DDT.

The analysis of fish tissue for the many DDT derivatives tends to be rather time-consuming; extensive clean-up is necessary in order to protect the sensitive electron capture detectors on the gas chromatograph. For many purposes a detailed analysis is not necessary, such as surveys concerned with trends in pesticide concentrations rather than with metabolic studies, and in these cases the analytical procedures can be simplified. To eliminate the need for elaborate clean-up procedures, the fish tissue can be destroyed effectively and rapidly by treatment with hot potassium hydroxide. A drawback with this procedure is, however, that DDT is dehydrohalogenated to DDE. Although this reaction occurs in a quantitative manner, it cannot be established with certainty what part of the analytical result for DDE represents DDT and what part represents metabolized DDT-DDE. In addition, DDD and other metabolites are destroyed. However, errors from this source are small since the concentration of DDD and other metabolites is generally low. As mentioned previously, the effect of storing frozen fish samples may lead to "disappearance" of DDT. While the method used to establish DDE levels is fully adequate for the present purpose, it should be recognized that the concentrations presented represent minimum values because of these potential sources of loss. It might be added that this method is used by many pesticide laboratories, including the United States Bureau of Commercial Fisheries, Ann Arbour, Michigan. In addition, this method is described in the official U.S. Department of Health Education and Welfare publication entitled "Guide to the Analysis of Pesticide Residues".

EXPERIMENTAL METHODS

The method used in this study consisted of the saponification of a suitable sample of fish tissue, subsequent separation of the DDE by partition into hexane clean-up of the extract by column chromatography and final separation and measurement of DDE by gas liquid chromatography

Fish tissue samples were homogenized by passing them three times through an electric meat grinder Suitable portions of the homogenate were then refrozen for storage For the saponification step, a 10-gm. aliquot of the homogenate was taken and boiled for approximately 30 minutes with 40 mls. of a 20% alcoholic potassium hydroxide solution. This treatment destroyed almost all fish tissue, converting it to a soapy, water soluble solution. DDT present was converted in the process to DDE.

Separation of the DDE from the majority of the saponification products was achieved by solvent extraction. The soapy solution was rinsed with distilled water into a 125-ml. separatory funnel with Teflon tap, and extracted with 3 x 15 ml. portions of hexane. The hexane extract which contained the DDE was made up to 50 mls in a volumetric flask. Final clean-up was carried out by column chromatography on Florisil. The column consisted of a 4" disposable Pasteur pipette, plugged at the lower end with glass wool. Into this was placed a 2" layer of activated Florisil, topped with 12 mm. of anhydrous sodium sulphate. One ml. of the hexane extract was run in, and the DDE eluted from the Florisil with 2 mls. of hexane. The eluate was collected in a 10-ml. graduated centrifuge tube, and the total volume made up to 5 mls.

The gas chromatographic separation and measurement step was carried out using an Aerograph 1520 series Gas Chromatograph fitted with electron capture detectors. The following column and operating conditions were used:

Column used	84" x 1/8" coupled stainless steel tube
Column Packing (on Chromosorb W.)	(1) 5 ft. of 5% QF-1, followed by (2) 2 ft. 10% SE-30.
Column temperature	180°C.
Injector temperature	190°C.
Detector temperature	185°C.

Range	EC.1
Attenuation	X 8.
Gas Flow	30 ml./min of nitrogen.

The instrument was calibrated with high purity p,p'-DDE. The p,p'-DDE calibration curve was linear over the range 0-2000 picograms. Retention time of p,p'-DDE was 5.3 minutes. Concentrations of DDE were established on the basis of original wet weight of sample.

Recovery studies from fish oil using this method indicated approximately 95% conversion and recovery of p,p'-DDT as p,p'-DDE. Samples in which high DDE levels were noted (over 40 ppm) were also run on thin layer chromatography as a confirmatory technique. Agreement with gas liquid chromatography results was good.

RESULTS AND DISCUSSION

A summary of the fish collected in each of the areas sampled is provided in Table 1. It is notable that most of the lake trout taken were large in size, 11 of the 15 fish weighing five pounds or more and seven of these weighing in excess of 14 pounds. It is believed that the smaller fish represent two-year old hatchery-reared fish that were introduced to the lakes in 1965.

Table 1. Summary of fish collected from Muskoka Lakes in September, 1966.

SPECIES	NUMBER OF SPECIMENS	LOCATION	WEIGHT RANGE (lbs.)
Lake Trout	6	L.Rosseau-Norway Pt.	6½ - 19¾
Whitefish	9	L.Rosseau-Norway Pt.	2½ - 6¾
Lake Trout	5	L.Rosseau-Treasure Is.	2¾ - 19¾
Lake Trout	1	L.Muskoka-Bracebridge	15¼
Lake Trout	3	L.Muskoka-Beaumaris Is.	5 - 21¼

Table 2 provides the results of DDE analyses for each individual fish. Using three tissues (muscle, gonad and liver) the average DDE content of the Lake Trout from Norway Point, Treasure Island and Beaumaris Island is 16.1 ppm, 17.7 ppm and 25.1 ppm respectively.

Table 2. DDE Concentrations of Individual Fish

SPECIES	WEIGHT (lbs)	LENGTH (cms)	SEX	Parts per Million DDE MUSCLE	GONAD	LIVER
				Lake Rosseau - Norway Point		
Lake Trout	6½	60.0	M	29.4	2.9	39.4
"	6¾	64.0	M	25.8	2.4	14.5
"	8½	64.5	F	23.5	5.4	3.5
"	14¼	79.0	F	28.9	7.7	2.9
"	17¼	66.5	M	49.4	10.0	4.0
"	19¾	89.0	F	32.3	6.6	1.4
				$\bar{x}$=31.6	$\bar{x}$=5.8	$\bar{x}$=10.9
White Fish	2½	48.0	M	16.7 - wholefish x.s.		
"	2½	48.5	M	2.4	2.5	*
"	2¾	51.0	M	2.6	4.2	*
"	3¼	55.5	M	23.7 - wholefish x.s.		
"	3½	54.0	F	24.3 - wholefish x.s.		
"	4¾	56.0	M	19.3	9.9	*
"	4¾	53.0	M	33.5 - wholefish x.s.		
"	5¾	65.5	M	24.4	16.9	*
"	6¾	65.5	M	19.8	16.9	*
				$\bar{x}$=13.7	$\bar{x}$=10.1	
				Treasure Island		
Lake Trout	2¾	52.0	M	17.0	3.5	58.2
"	3¼	53.5	M	4.3	22.1	27.5
"	3½	55.0	M	27.8	1.9	17.0
"	4.0	57.0	M	18.8	4.0	18.8
"	19¾	93.5	M	29.3	2.0	18.3
				$\bar{x}$=19.9	$\bar{x}$=6.7	$\bar{x}$=27.0
				Lake Muskoka - Bracebridge		
Lake Trout	15¼	80.2	M	110.0	10.3	49.3
				Beaumaris Island		
Lake Trout	5.0	65.0	F	14.6	8.0	9.5
"	18¼	85.0	M	74.2	6.7	30.3
"	21¼	86.0	F	71.5	15.9	4.3
				$\bar{x}$=53.5	$\bar{x}$=10.2	$\bar{x}$=11.4

* Tissue not analysed

The whitefish population in the area of Norway Point shows a lower average DDE concentration than does the trout population from the same area. The muscle tissue from the Norway Point whitefish averaged 13.7 ppm DDE whereas the lake trout muscle averaged 31.6 ppm DDE. However, the average DDE content of whitefish gonads was found to be 10.1 ppm and lake trout gonads averaged 5.8 ppm.

Premdas (1963) states that under-yearling atlantic salmon (Salmo salar), when exposed to 1 ppm C14-DDT for 5 minutes, showed highest concentrations in the liver, gonad, spleen, gills, heart, kidney, and swim bladder, lesser amounts in the stomach, gut, brain and spinal cord, and least amounts in the skin, bone, and muscle. The distribution of DDE in trout collected from the Muskoka Lakes was quite dissimilar. The gonad tissue of the trout showed the lowest average concentration of DDE, the liver contained intermediate concentrations, while the average concentration in the muscle exceeded levels in the gonads by as much as nine times. Anderson and Everhart (1966) point out that DDT contents were related to condition of the fish, i.e. fat fish contained higher concentrations of the pesticide than lean individuals.

The relationship of DDE to loss of reproductive capacity in lake trout is not known. DDE concentrations could represent DDT taken up by the fish and subsequently metabolized, or in part might represent direct accumulation through ingestion of small fish and other food items containing the insecticide in the metabolized form. Since all of the DDT was converted to DDE by the analytical procedure used, direct comparisons with other studies in which DDT concentrations were related to declining fish stocks cannot be made.

Nonetheless, the information provided in Table 3 following, which provides the results of three complete determinations (DDT, DDD and DDE) for two muscle samples and one gonad sample, indicates that the percentage of DDT relative to total DDE was 81%, 70% and 83% respectively. Also, the table indicates the close agreement for total DDE values obtained by the two laboratories.

Table 3. DDT, DDE and DDD determinations for lake trout samples. *

SAMPLE	PESTICIDE	FDD RESULTS (ppm)	TOTAL DDE(FDD)	OWRC
Muscle (L.Muskoka)	DDT	72.9	90.9	86
	DDD	8.2		
	DDE	18.0		
Muscle (L. Rosseau)	DDT	19.5	27.7	29.3
	DDD	4.5		
	DDE	8.2		
Gonads (L.Rosseau)	DDT	3.9	4.7	5.4
	DDD	0.3		
	DDE	0.8		

* these determinations were completed by the Federal Food and Drug Directorate, Toronto Regional Laboratory.

Assuming the same significance for DDT concentrations in the gonads as Burdick et al. (1964) described for lake trout eggs from Lake George, New York State (mortality of young fry associated with 2.93 ppm in ether extract of the eggs) it would appear, if our three percentages for DDT are truly representative, that none of the five female lake trout listed in Table 2 would be capable of reproducing without subsequent fry mortality developing. Furthermore, it should be stressed that the concentrations expressed by Burdick and his co-workers are in terms of the ether-extracted oils from the eggs while our values are based on total wet weight of gonad samples. Included in the data provided in the New York paper is a table which indicates that DDE concentrations in oils extracted from the eggs are 10 to 15 times the concentrations based on total weight of egg tissues. Application of a conservative factor of 10 to our results to render a more valid comparison would produce extremely high values for the ovaries of the five female lake trout, ranging from 54 to 159 on the basis of presumed oil content. This projection highlights the seriousness of prevailing levels of DDT and its metabolites in lake trout from Lake Muskoka and Lake Rosseau.

Although a number of assumptions are made in the above comparison, the high values obtained for DDE point up the need for

additional information on concentrations of DDT in lake trout eggs and the survival of fertilized eggs taken from fish in the Muskoka Lakes system.

The Food and Drug Directorate have set a residue tolerance of 7 ppm for DDT in the fat of certain meats. So far, residue tolerances for various pesticides in fish sold for human consumption have not been established. In the absence of an established limit, foods having a detectable concentration may not be sold. Since residue tolerances take into account the frequency of use in the diet, the transferance of tolerances established for meat to fish (especially game fish) may not be valid. However, the extremely high levels of DDE obtained in the flesh of the lake trout analyzed raises the question of whether these fish are suitable for human consumption.

ACKNOWLEDGEMENTS

The co-operation of fish and wildlife personnel of the Parry Sound Forest District of the Department of Lands and Forests in obtaining fish for DDE analyses was much appreciated. Mr.Carman Douglas, Fish and Wildlife Supervisor, Parry Sound District,provided information on fish species composition and other facts pertaining to the fishery of the Muskoka Lakes.

REFERENCES

Anderson, Richard B., and W. Harry Everhart. 1966. Concentrations of DDT in landlocked salmon (Salmo salar) at Sebago Lake, Maine. Trans. Am. Fish. Soc., 95(2): 160 - 164.

Bridges, W. R., B. J. Kallman, and A. K. Andrews. 1963. Persistence of DDT and its metabolites in a farm pond. Trans. Am. Fish. Soc., 92(4) : 421 - 427.

Burdick, G. E., E. J. Harris, H. J. Dean, T. M. Walker, Jack Skea, and David Colby. 1964. The accumulation of DDT in lake trout and the effect of reproduction. Trans. Am. Fish Soc., 93(2) : 127-136.

Cope, Oliver B. 1961. Effects of DDT spraying for spruce budworm on fish in the Yellowstone River system. Trans. Am. Fish Soc., 90(3) : 239 - 251.

Faobichon, D. J. and P. W. Saschenbrecker. 1967. Dechlorin[illegible] [illegible]sen blood. Science, 156(3775) : 663 - 665.

Henderson, C., Q. H. Pickering, and C. M. Tarzwell. 1959. Relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish. Trans. Amer. Fish Soc., 88(1) : 23 - 32.

Hill, David W., and Perry L. McCarty. 1967. Anaerobic degradation of selected chlorinated hydrocarbon pesticides. Jour. Water Poll. Control Fed., 39 (8) : 1259 - 1277.

Ide, F. P. 1956. Effect of forest spraying with DDT on aquatic insects of salmon streams. Trans. Amer. Fish Soc., 86(1) : 208-219.

Mack, G. L., S. M. Corcoran, S. D. Gibbs, W. H. Gutenmann, J. A. Reckahn, and D. J. Lisk. 1964. The DDT content of some fishes and surface waters of New York State. New York Fish and Game Journal, 11(2) : 148 - 153.

Premdas, F. H., and J. M. Anderson. 1963. The uptake and detoxification of C^{14}-labelled DDT in Atlantic salmon, Salmo salar. J. Fish. Res. Bd. Canada, 20(3) : 827 - 837.

MOE/MUS/PRE/AOZP
Ontario Water Resources Co
A preliminary report
on the accumulation aozp
c.1 a aa